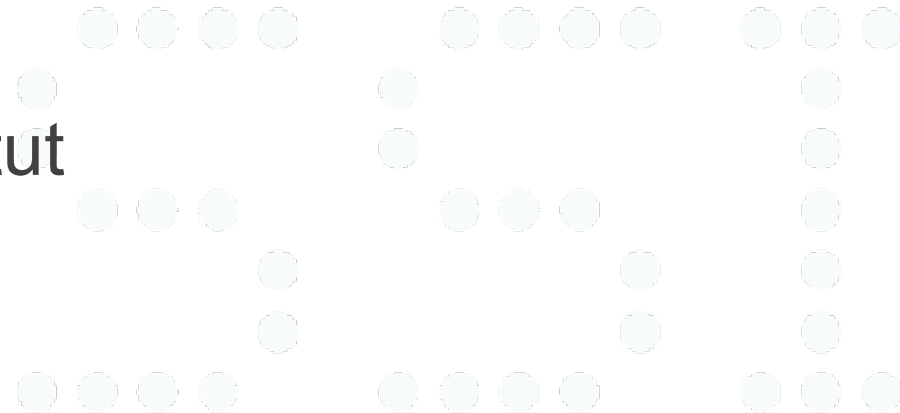




## URINARY TRACT INFECTIONS AND BIOFILMS

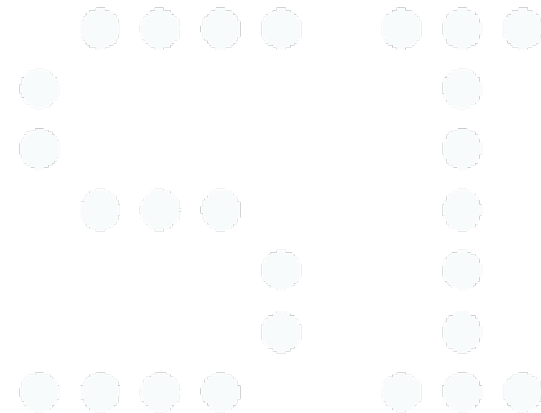
- Karen A Krogfelt
- Professor
- Statens Serum Institut

kak@ssi.dk

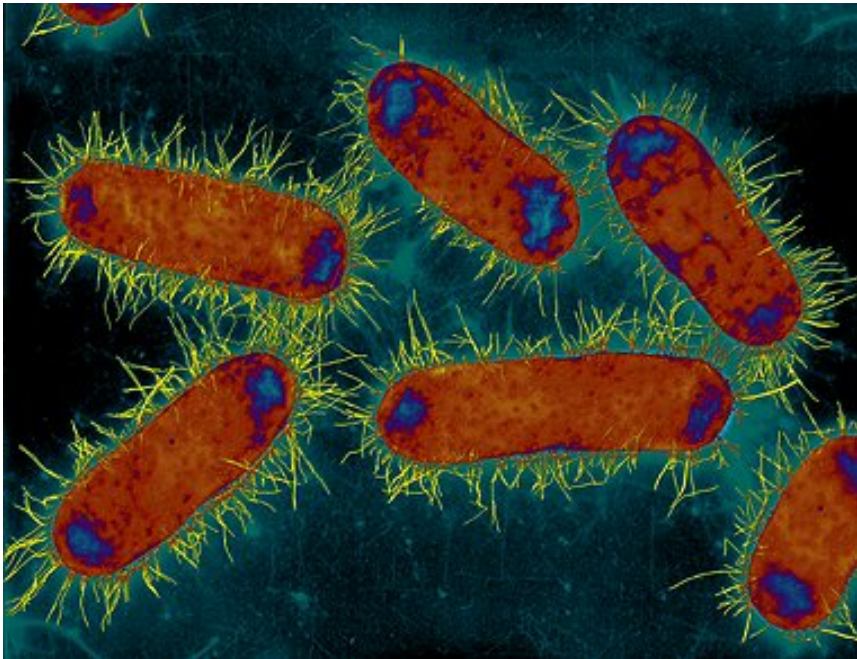


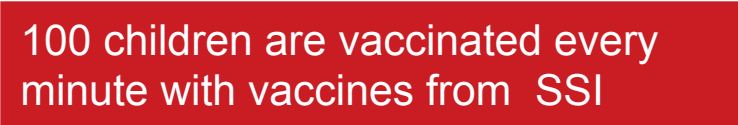



## The National Institute for Health Documentation and Disease Control




- ❖ SSI is the national institute for health documentation and e-health
- ❖ SSI maintains detection, surveillance, research, prevention and control of infectious diseases, biological threats and congenital disorders



- Established in 1902
- Ministry of Health Agency and State 

100 children are vaccinated every minute with vaccines from SSI
- 1400 employees
- Total annual turnover: 200m EUR 

10m vials = 40-80 m vaccine dosis  
0.5m diagnostic analysis
- Export sales: 53m EUR 

1.5 international publication per day
- Government subsidy: 16m EUR
- Research funds: 15m EUR



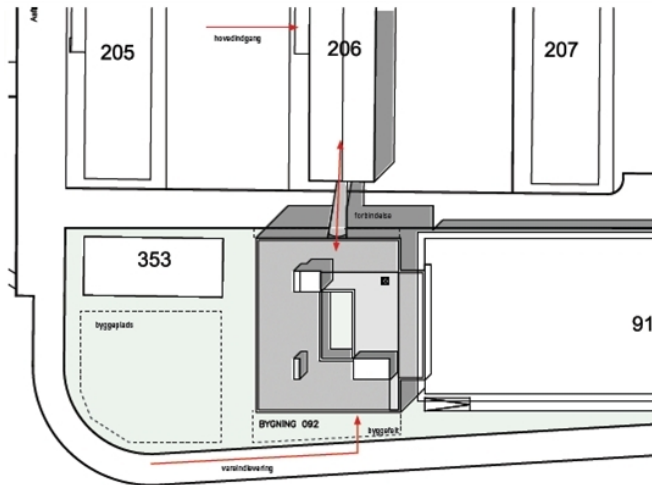
1902 Anti-diphtheria serum  
Typhus- and syphilis laboratory analysis



1910 Anti-tetanus serum  
Influenza pandemi



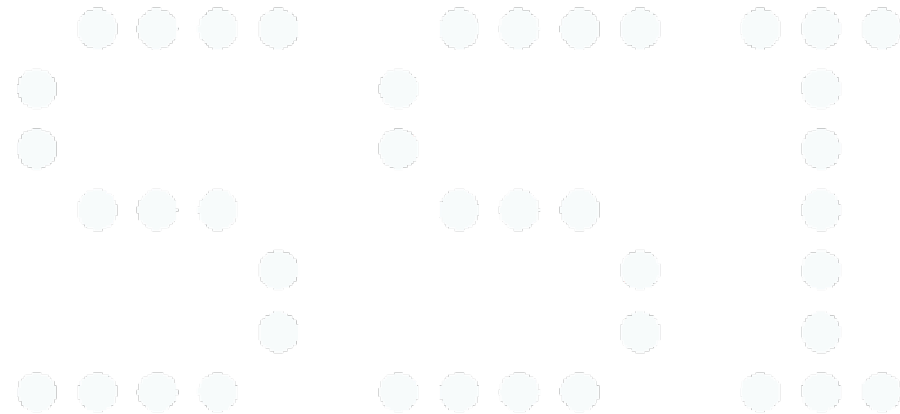
# THE DANISH NATIONAL BIOBANK 2012



- Ensures data regarding the health status of the Danish population, including financial and quality control data
- Ensures national frameworks and standards for e-Health
- Surveillance and control of infectious diseases and congenital disorders
- Reference laboratories for infectious, autoimmune, congenital and genetic diseases
- National supply and export of vaccines and other biological products through production and procurement
- Centre for Biosecurity and Biopreparedness
- Research and development in the Institute's areas of activities

# CHARACTERIZATION OF *KLEBSIELLA PNEUMONIAE* VIRULENCE FACTORS INVOLVED IN CATHETER ASSOCIATED URINARY TRACT INFECTIONS

Carsten Struve  
Casper Scroll  
Steen Stahlhut  
Martin Steen Mortensen





# ***KLEBSIELLA PNEUMONIAE***

- ❖ Gram negative
- ❖ Non-motile
- ❖ Rod-shaped
- ❖ Enterobacteria
- ❖ Usually encapsulated
- ❖ Environment (water, soil, plants)
- ❖ Mucosal surfaces of mammals
- ❖ Opportunistic pathogen
- ❖ Nosocomial
  - Urinary tract infection (UTI)
  - Catheter associated UTI
  - Pneumonia
  - Sepsis
- ❖ **Recently:** Community acquired *K. pneumoniae* causing pyogenic liver abscess

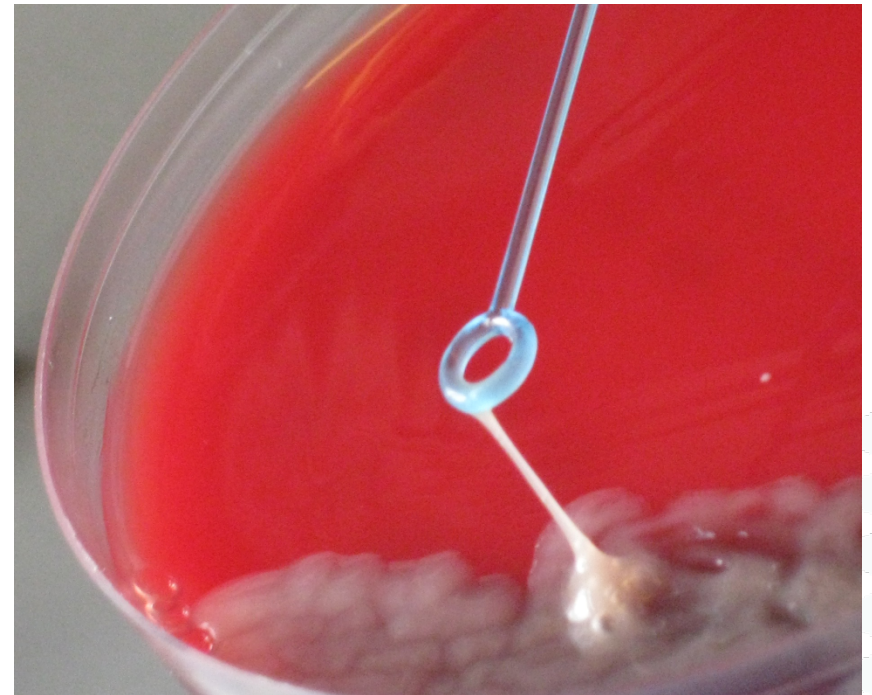


Photo by Carsten Struve

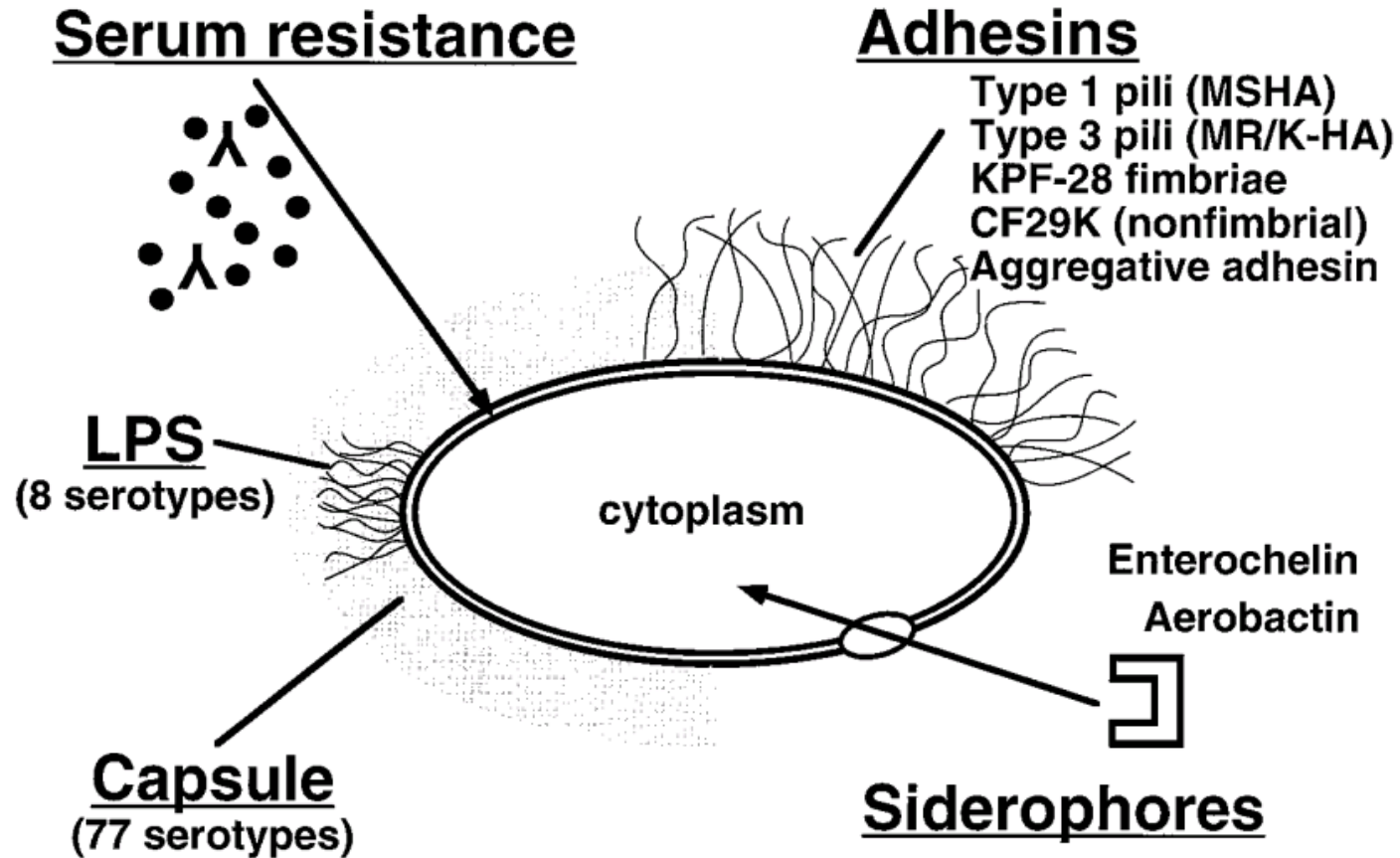
## **Opportunistic pathogens**

- 3-7 % of nosocomial bacterial infections
  - Approx. 10 % of all UTI
- 3<sup>rd</sup> most common cause of bacteremia
  - mortality rates from 20% to 55 %
- Highly virulent strains has caused community-acquired pyogenic liver abscesses

## **Strains highly diversified regardless of source**

- Except strains from pyogenic liver abscesses

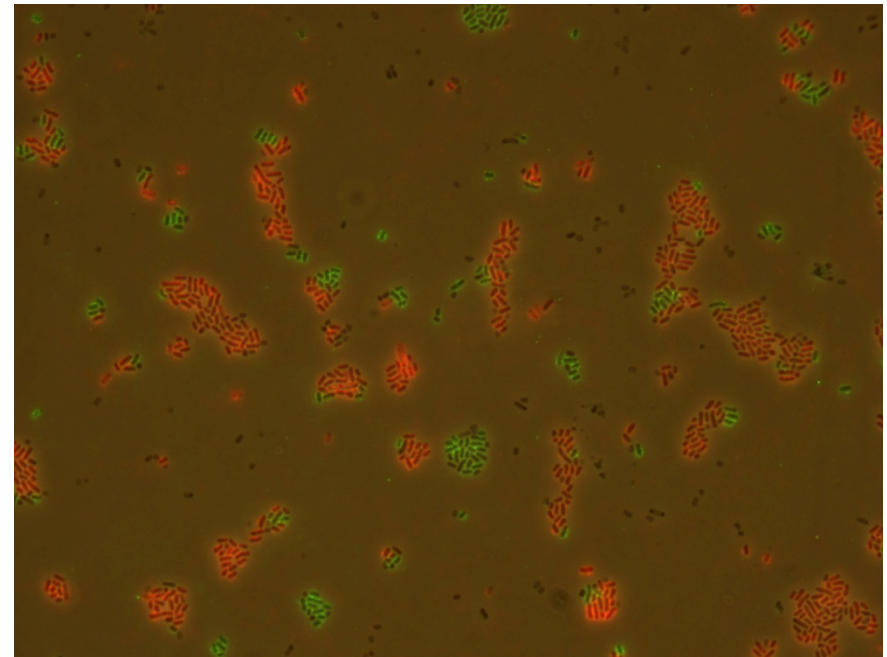
## **Antibiotic resistance is of increasing concern**





## ADHESION FACTORS OF *K. PNEUMONIAE*

- ✧ Type 1 Fimbriae
  - Well studied
  - Found in *Enterobacteriaceae*
  - UTI
- ✧ Type 3 Fimbriae
  - Found mainly in *Klebsiella spp.*
  - Biofilm
  - Unknown receptor



*K. pneumoniae* fimbriae expression

Red: Type 1 fimbriae expression

Green: Type 3 fimbriae expression

**Found in many different *Enterobacteriaceae*.**

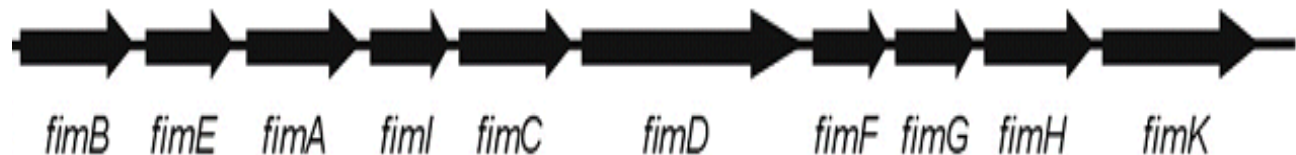
- 7 nm wide and 0.2 to 2.0  $\mu\text{m}$  long

**Phase variable promoter**

- Individual cells to switch between fimbriated and non-fimbriated state

**Important virulence factor during UTI**

- Influence in other types of infections not thoroughly investigated
- Do not affect virulence in infection of lungs and intestines of mice



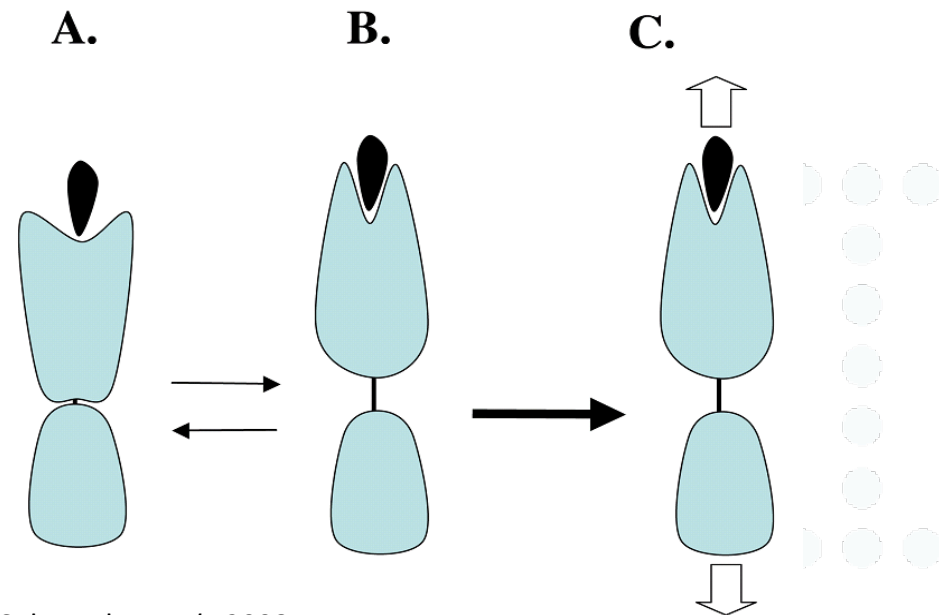
Schematic representation of type 1 fimbrial (*fim*ABCDEHFK) gene arrangements in *K. pneumoniae* c3091 (Struve *et al.*, 2009)

## FimH comprised of two domains

- Mannose-binding lectin domain, aa 1 to 156,
- Pilin domain, aa 160 to 279

## Catch bonds

- Becomes stronger under increasing shear forces
- Allows FimH to bind loosely when shear forces are low



Sokurenko *et al.*, 2008



**First described in *Klebsiella spp.***

- Shown to only agglutinate tannin treated erythrocytes

**Exist in several distinct variants**

- Antigenic diversity in different genera of enterobacteria

**Binding pocket nor the receptor identified**

- Indication of catch bond binding

**Adhesion and biofilm formation**



Schematic representation of type 3 fimbrial (*mrkABCDF*) gene arrangements in *K. pneumoniae* c3091 (Struve *et al.*, 2009)

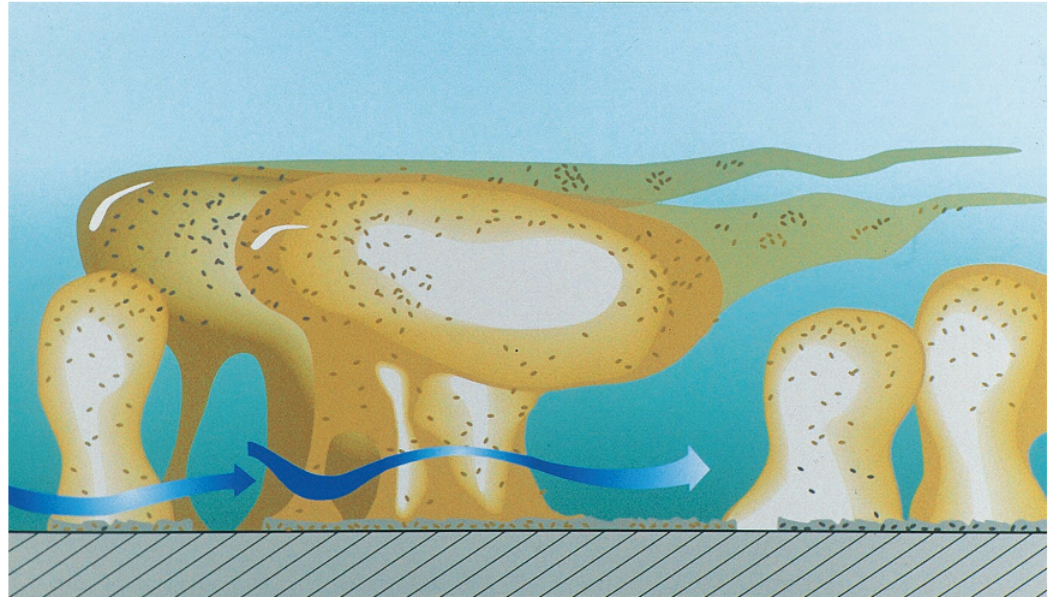


## Protect bacteria from

- Antibiotic agents
- Disinfectants
- Host immune system

## Nosocomial burden

- Increased treatment length
- Increased risk of treatment failure
- Increased treatment costs for hospitals



Donlan & Costerton, 2002



I

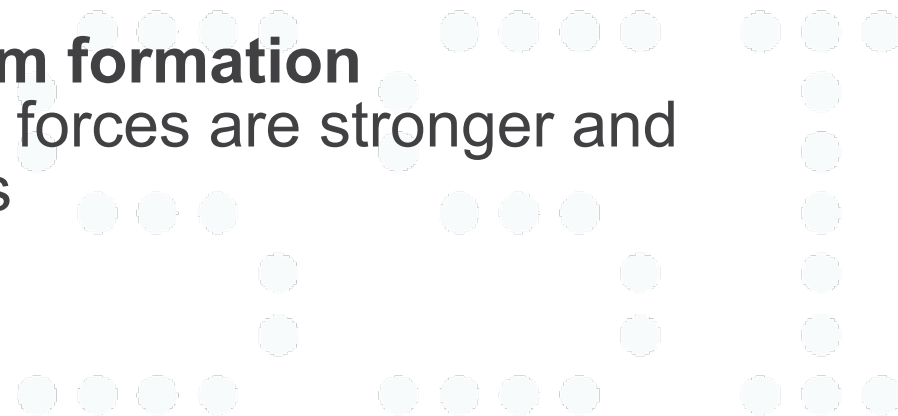
**initiated by planktonic cells adhering to surfaces**

**Exopolysaccharides used to form matrix structure**

**Bacterial cells in biofilms are phenotypically altered**

**High shear forces induce biofilm formation**

- Biofilms formed at high shear forces are stronger and resistant to mechanical stress



1 mm

5  $\mu$ m

# CATHETER ASSOCIATED URINARY TRACT INFECTIONS (CAUTI)

## Indwelling catheters are widely used

- Hospitals and nursing homes
- Up to 50 % of patients are catheterized
- Increases risk threefold compared to non-catheterized patients

## CAUTI in 25 % of patients with indwelling catheter >7 days

- Risk increases 5 % daily

## 26 % of indwelling urinary catheters colonized by *Klebsiella spp.*

- > 67% of these were *K. pneumoniae*

## Biofilms has a huge influence on development of CAUTI

## **Commonly used to investigate effect of mutations**

- Growth rates, growth on specific substrates, biofilm formation.

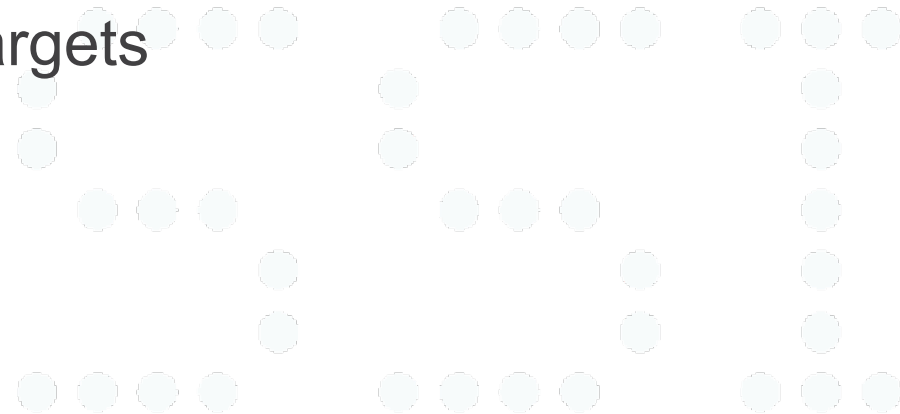
## **Allows for large scale screening of large mutant or strain libraries**

- Used in several studies

## **Many studies include coating with human extracellular matrix**

- Present relevant binding targets
- Increases transferability

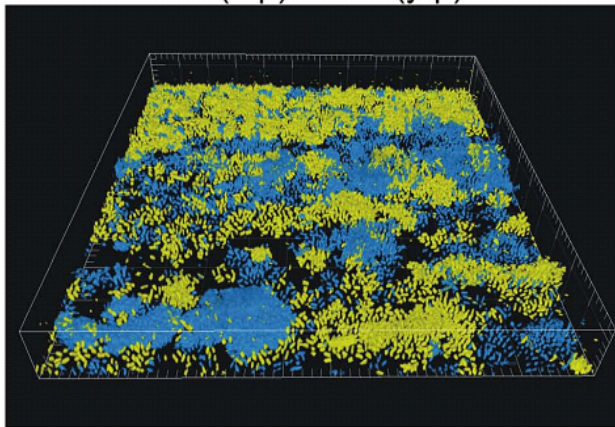
## **Coating**



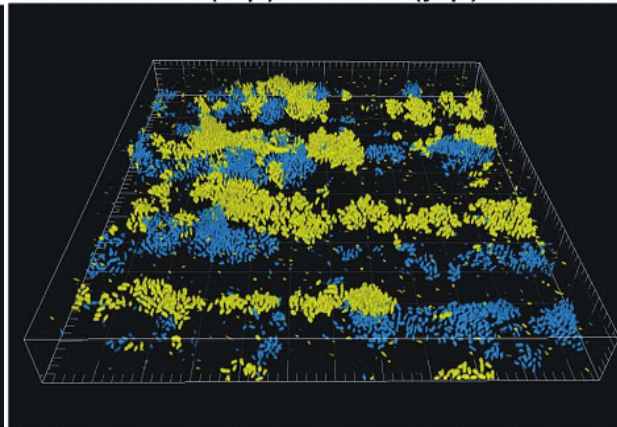


Used to investigate biofilm formation  
Direct on-line microscope examination  
Laser scanning confocal microscopy  
(LSCM)

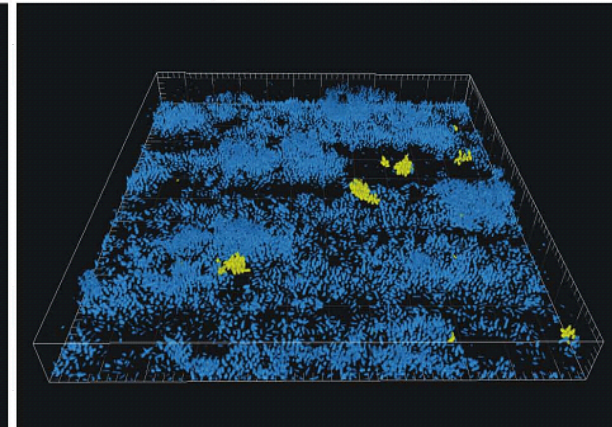
Wt(cfp) vs Wt(yfp)



Wt(cfp) vs  $\Delta fim$ (yfp)



Wt(cfp) vs  $\Delta mrk$ (yfp)



Schroll et al. 2010



## Used to investigate bacterial cell adhesion and invasion

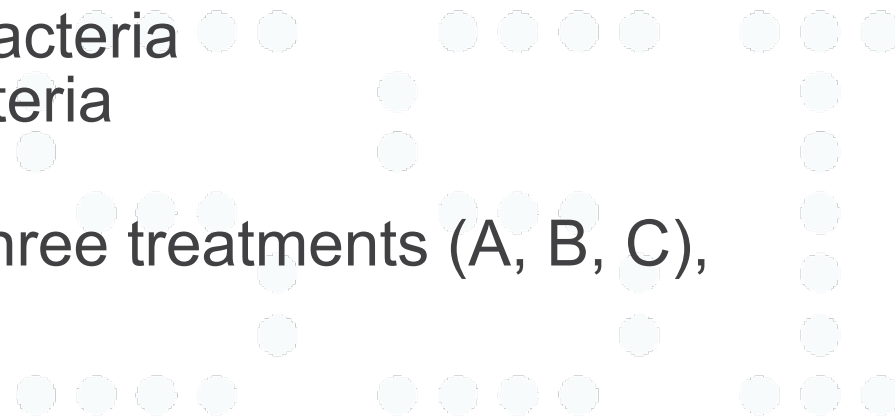
- Ability of *K. pneumoniae* C3091 to invade human cell lines
- Human bladder epithelial cells, 5637 (ATCC HTB-9)

## Monolayer of cell line in cell trays

- three separate cell trays
  - **A:** TOTAL bacteria
  - **B:** CELL-ASSOCIATED bacteria
  - **C:** INTRACELLULAR bacteria

## Procedure

- Grow monolayer, incubate, three treatments (A, B, C), plate



## Used to investigate bacterial virulence factors *in vivo*

- Rodents are widely used as laboratory animals
- Several models of UTI in rodents

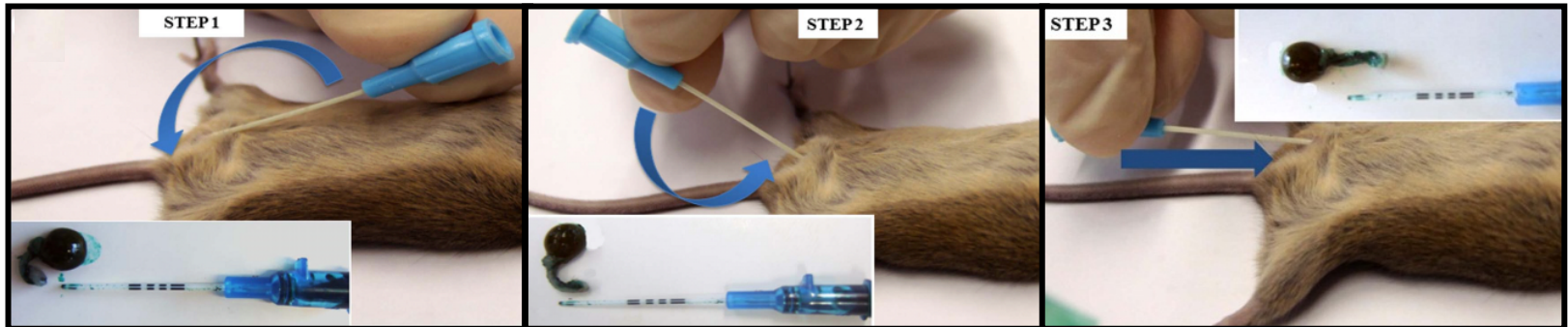
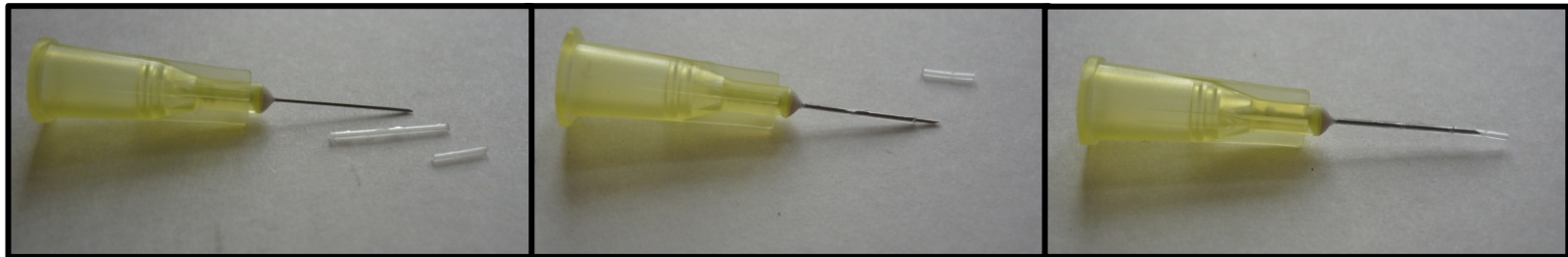
## Foreign bodies cause major physiological changes in the bladder

### Procedure

- Catheterization, inoculation, euthanization, homogenization, plating



# CATHETERS AND CATHETERIZATION

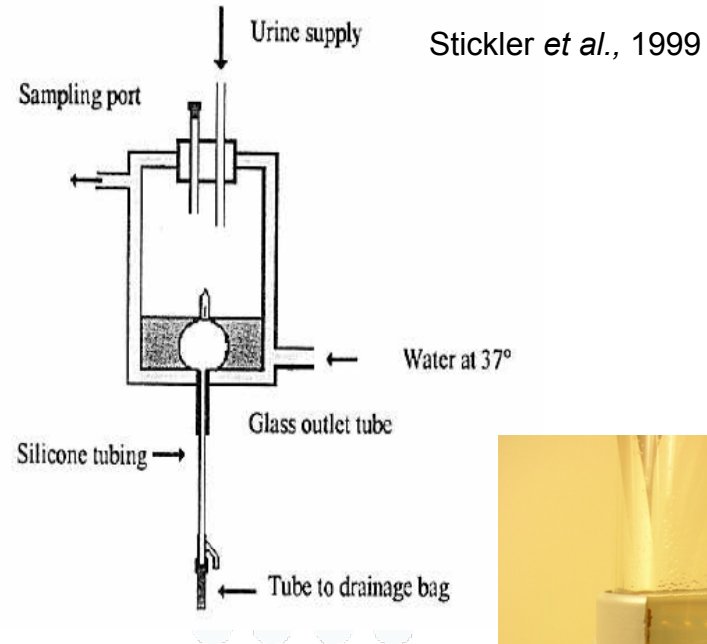


Reis *et al.*, 2011

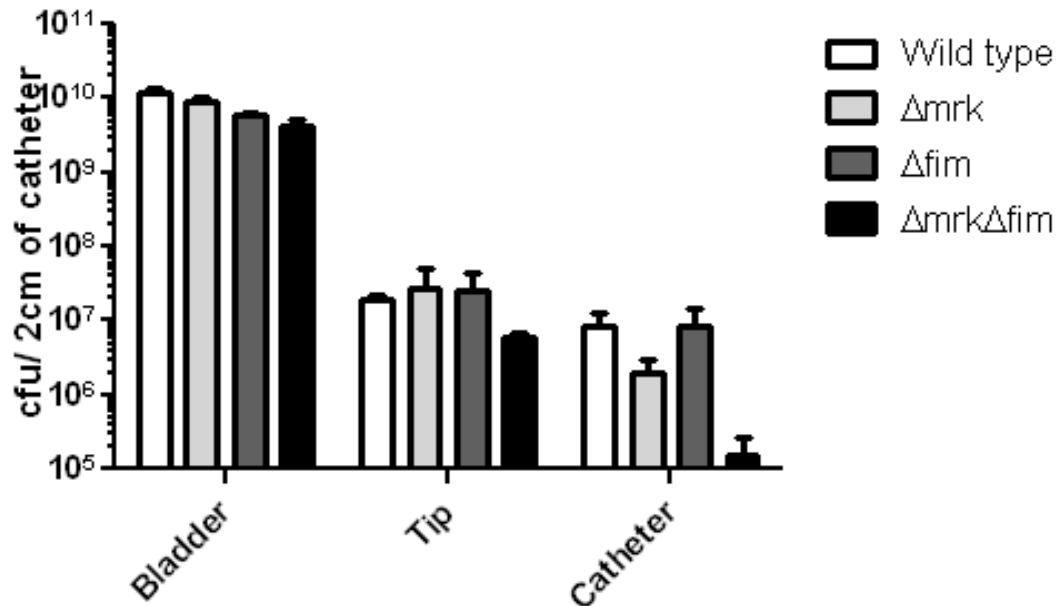
# HUMAN-LIKE CATHETERIZED BLADDER MODEL



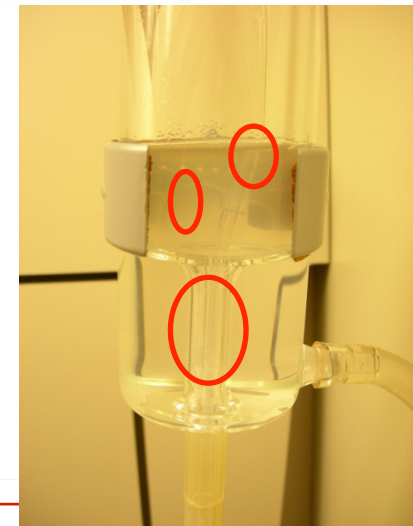
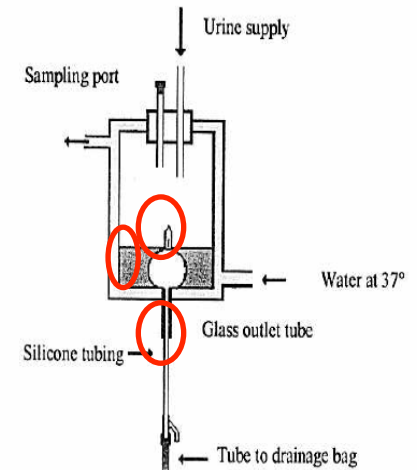
Stahlhut ,SSI 2010



# ROLE OF TYPE 1 AND TYPE 3 FIMBRIAE IN BIOFILM FORMATION ON URINARY TRACT CATHETERS IN HUMAN URINE



Stickler *et al.*, 1999



Method	Advantage	Disadvantage
Biofilm microtiter plate assay	Fast screening Multiple strains Coating	Low transferability
Biofilm flow chamber assay	Qualitative In-line Coating/mammalian cells	Complicated technique Limited availability
Cell adhesion and invasion assay	Improved transferability Mammalian cells	Facilities and expertise Time Variable on a daily basis
Murine CAUTI model	Improved animal model Simulate CAUTI <i>in vivo</i>	Cost Complexity of model Mice dropping catheters



## **Lack of correlation between *in vitro* and *in vivo* results**

### ***In vitro* experiments can indicate a virulence factor**

- Increased or decreased ability to cause infection

### ***In vivo* experiments to determine significant effect for infection**

- Animal models to simulate human infection



## **Statens Serums Institut (SSI)**

- Ph.D. Carsten Struve
- Ph.D. Steen Stahlhut
- MSc Martin Mortensen

## **Clegg lab**

- Professor, Ph.D. Steven Clegg
- M.Sc. Caitlin Murphy
- B.Sc. Sarah Zeiner
- B.Sc. Brett Dwyer

